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Antipyretic Effects of Earthworm Extracts on Peptone-Induced Fever in Mice

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ABSTRACT

Fever, defined as a body temperature elevation exceeding 37.5°C, is a hallmark of numerous pathological conditions. *Lumbricus rubellus*, commonly known as the earthworm, has been implicated in the amelioration of fever, particularly in response to infection by *Salmonella enterica* serotype *Typhi*. Commercially available earthworm-derived formulations are purported to exert a broad-spectrum antipyretic effect, extending beyond bacterial etiologies. This investigation employed a controlled, before-and-after experimental design to elucidate the antipyretic potential of earthworm extracts on mice subjected to peptone-induced hyperthermia. Upon verification of a peptone-induced body temperature increase above 37.5°C in mice, interventions were administered orally across three cohorts: a test group receiving earthworm extract suspension, a positive control group provided with paracetamol syrup, and a negative control group receiving a Na-CMC suspension. The primary outcome, alterations in body temperature, was statistically evaluated using the Kruskal-Wallis and Mann-Whitney tests. The Kruskal-Wallis test, utilized for assessing variance across multiple groups, yielded a p-value of 0.000 within the test group, indicative of a significant deviation in mean body temperature post-administration of the earthworm extract. Subsequent analysis with the Mann-Whitney test, comparing the degree of temperature reduction at 60 minutes post-intervention between the earthworm extract and paracetamol syrup groups, manifested a p-value of 0.017. This result suggests a statistically significant disparity, with the most pronounced temperature decrease observed in the earthworm extract cohort, followed by the paracetamol group. The Na-CMC administered cohort displayed no significant antipyretic effect. Observational data indicate that the antipyretic effect of earthworm extract significantly induced acetaminophen in an induction hyperthermia model.

1. INTRODUCTION

Fever, manifesting as an elevation in body temperature beyond the normal threshold of 37.5°C, serves as a ubiquitous physiological response to both infectious and non-infectious stimuli [1, 2]. Fever is a defense mechanism or immune response to maintain body homeostasis. In normal conditions, fever lasts between two to three days, but if more than that, it could indicate an infection in the body [3].

Fever is a common symptom of many diseases, such as viral infections, parasitic infections, bacterial infections, autoimmune diseases, cancer, inflammatory bowel disease, and liver disease [4, 5]. Fever may manifest as a response to specific pharmacological agents or immunizations and can also serve as an indicator of non-infectious disease states. In addition, fever does not always have a known cause, which is called idiopathic fever. In Indonesia, idiopathic fever is the most common symptom in primary care, accounting for approximately 45% of visits [6]. Fever due to infection is also common, as evidenced by Indonesia's 2013 health profile which revealed that the number reached 112,511 cases with a total of 871 deaths [7]. Several infections with the main symptom of fever, such as Dengue Hemorrhagic Fever (DHF) (A91) and typhoid fever (A01.0) are common in Indonesia. In 2015, the World Health Organization (WHO) recorded Indonesia as the country with the highest dengue cases in Southeast Asia. Based on data from the Ministry of Health of the Republic of Indonesia [8], dengue reached 95,893 cases. In addition, the incidence of typhoid fever is also quite high, ranging from 350-810 cases per 100,000 population [9]. Based on these cases, many efforts to treat fever have been developed ranging from chemical drugs to traditional medicines such as earthworms (*Lumbricus rubellus*) [10, 11].

Earthworm (*Lumbricus rubellus*) is a traditional medicine from animal materials that is used to treat fever, especially typhoid fever caused by Salmonella thypi bacteria. According to Dewi et al. [12], earthworms have properties in the form of phenols and antioxidants. In addition, Istiqomah et al. [13] said that earthworms are rich in peptides, such as lumbricin. Lumbricin is composed of complete amino acids, especially proline as a secondary metabolite and in vitro has been shown to have a broad-spectrum antimicrobial activity that can inhibit gram-positive bacteria, gram-negative bacteria, and several fungi such as *Escherichia coli, Salmonella, Staphylococcus aureus, Streptococcus aureus*. Lumbricin works by altering membrane permeability so that microbial cells are lysed [13, 14]. Previous researches by Rizky et al. [14] and Samatra et al. [15] have suggested the potential of *Lumbricus rubellus* extract in inhibiting the proliferation of *Salmonella typhi*, thereby mitigating febrile symptoms associated with the infection.

Antipyretic activity testing can be done by three methods: lipopolysaccharide induction, yeast induction, and peptone induction. This study used the peptone induction method which is easily available and non-toxic. Peptone compound is one of the exogenous pyrogens that can cause fever. Peptone that enters the body is recognized as an antigen or foreign substance because it contains lipopolysaccharide (LPS) which is a component of the cell wall of gram-negative bacteria. This causes the immune system of the experimental animals to become active. Peptone will then stimulate phagocytic activity that triggers the release of endogenous pyrogens such as IL-1, IL-6, IFN, and TN. Peptone will stimulate the hypothalamus to form prostaglandins. The production of many prostaglandins causes the temperature threshold to be higher, resulting in fever. Peptone induction generally uses mice and after the temperature rises, measurements can be made for the antipyretic activity of the test compound. Peptone is a hydrolyzed protein, potent as a fever trigger, and has no toxic properties. Activity testing will be carried out by inducing peptone subcutaneously to increase the body temperature of experimental animals. Induction is done subcutaneously so that the release or absorption of peptone is slowed down so that the duration of action becomes longer. This aims to ensure that the decrease in temperature is not caused by peptone levels that have decreased or no longer exist in the body but because of the antipyretic effect of the test solution [13-15].

The in vivo antipyretic activity of *Lumbricus rubellus* extract in peptone-induced hyperthermia in male mice (*Mus musculus*) remains to be substantiated with empirical data for non-typhoidal fevers. Research on earthworms (*Lumbricus rubellus*) as a fever-reducer in typhoid fever conditions has been widely done, however community also use earthworms as a fever-reducer in nontyphoid fever. In this case, no researchers have tested the antipyretic effect when the fever is not caused by *Salmonella thypi* or nontyphoid bacterial infection. Therefore, this research is important and will contribute to the manufacture of biomedicine with earthworm extract.

The investigation is predicated upon two hypotheses: the primary alternative hypothesis (Ha) posits a difference in the mean body temperature of male mice induced by peptone before and after the administration of *Lumbricus rubellus* extract at various intervals (30, 60, 90, and 120 minutes). The secondary Ha anticipates a differential impact on body temperature between mice treated with *Lumbricus rubellus* extract and those administered paracetamol syrup, particularly at the time point correlating with the most pronounced temperature reduction. The objectives are twofold: to ascertain the differential in mean body temperatures pre- and post-administration of *Lumbricus rubellus* extract at specified time intervals, and to compare the antipyretic effects of the earthworm extract against paracetamol syrup at the peak of temperature decline.

2. METHOD

The type of research used is a correlation with before-and-after design with experimental group control to determine the activity of earthworm extract (*Lumbricus rubellus*) on reducing the temperature of male mice (*Mus musculus*) induced by peptone and then analyzed using the comparative method. The sample selection used the purposive sampling technique, which is sampling with certain considerations according to the desired criteria to determine the number of samples to be studied. The inclusion criteria in this study were male mice weighing 20-30 grams, 2-3 months old, healthy, normal activity, had a body temperature > 37°C after peptone induction and had never been used for other experimental studies, with exclusion criteria for mice that died or became ill during the study.

Before treatment, mice were placed in a quiet place to avoid stress and fasted for eight hours but still given water. Male mice (*Mus musculus*) were induced with peptone solution of 5% subcutaneously and then measured body temperature rectally at T60 as a pretest result, then given an intervention according to the group. After the intervention, the body temperature (T) of male mice was again measured at T30; T60; T90; and T120°C as the post-test result. This study used a before-after with control group design with a total of 27 male mice (*Mus musculus*) that has been calculated using Federer's formula. The mice were divided into three groups. The test group was given a suspension of earthworm extract (*Lumbricus rubellus*) 38 mg/20 gBB mice orally, the comparison control group was given paracetamol syrup 2 mg/20 gBB mice orally, and the negative control group was given a 0.5% Na-CMC suspension orally. The flow of the activity test can be seen in Figure 1.

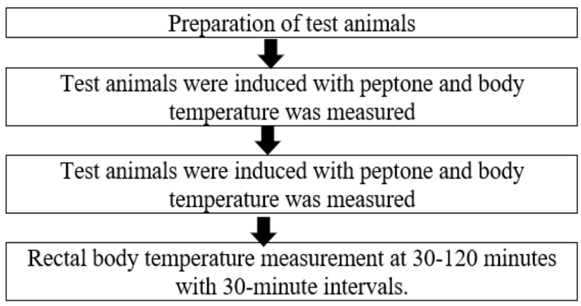

Figure 1. Flow of activation test application

The independent variable in this study is the suspension of earthworm extract, while the dependent variable is the body temperature of male mice (*Mus musculus*). Paracetamol syrup and Na CMC suspense are comparative controls.

Data analysis used the Kruskal-Wallis test to determine the difference in mean body temperature of male mice induced by peptone before and after being given a suspension of earthworm extract. Then to determine the position of earthworm extract and paracetamol syrup, the decrease in body temperature of male mice at T60 after being given the intervention was analyzed using the Mann-Whitney test. Post Hoc tests were conducted to determine the most significant temperature changes between minutes 30, 60, 90, and 120.

3. RESULTS AND DISCUSSION

The activity test aims to determine the antipyretic effects of earthworm extracts (*Lumbricus rubellus*) on peptone-induced fever in mice (*Mus musculus*). Before treatment, mice were not fed for 8 hours and then given peptone induction. The mice were given peptone induction subcutaneously according to the preliminary test procedure then body temperature was measured at the 60th minute which was recorded as T0. All test animals experienced an increase in body temperature of more than 0.60°C so that it can be stated that the peptone used is effective as a fever inducer. After being declared feverish, 0.5% Na-CMC suspension was given to as much as 0.2 mL/20 gBB mice orally. The negative control group, which is given the Na-CMC suspension intervention, needs to be done first. This is because Na-CMC suspension is used as a suspending agent in earthworm extract so to avoid bias in the data obtained, the negative control group is tested first. After the Na-CMC suspension is declared to have no antipyretic activity, the suspension of earthworm extract can be made. The absence of antipyretic activity in the Na-CMC suspension is indicated by the mice's body temperature which continues to increase. Through the results obtained, 0.5% Na-CMC suspension can be used as a suspending agent in earthworm extract. The suspension of earthworm extract was then administered at a dose of 38 mg/20 gBB mice orally. Comparative data on the mean body temperature of mice in the administration of 0.5% Na-CMC suspension and *Lumbricus rubellus* extract suspension can be seen in Table 1 and Figure 2.

Based on the temperature measurement results (Figure 2 and Table 1), the given of earthworm extract suspension can decrease the body temperature of male mice. The antipyretic activity of earthworms is caused by several compounds, such as coelomycetes. According to Engelmann et al. [16], coelomycetes is a fluid from coelomic cells containing lysozyme which plays a role in phagocytic activity and can increase the body's immunity. This is in line with Alesci et al. [17] research which states that coelomycetes are an innate immunity system and play a role in phagocytosis and opsonization activities in worms of Annelids phylum. Coelomycetes will stimulate the binding of antigens with antibodies into one large particle to facilitate the phagocytosis process. Then the anti-inflammatory cytokine IL-10 will be released and cause a state of homeostasis, resulting in a decrease in body temperature [18]. Apart from coelomycetes, based on the results of phytochemical screening carried out by Deri et al. [19], earthworms tested positive for alkaloids. Alkaloids can reduce fever by inhibiting the cyclooxygenase enzyme so that the formation of prostaglandins as mediators of increasing body temperature will be inhibited.

Table 1. Mean body temperature of mice in the activity test in 2023

Intervention	Average of Temperature (°C)					
	Early	T0	T30	T60	T90	T120
Suspension Na-CMC 0.5%	37.18 ±0.18	37.87 ±0.17	37.93 ±0.17	38.11 ±0.16	38.14 ±0.19	38.20 ±0.25
Lumbricus rubellus extract suspension	37.15 ±0.34	38.11 ±0.37	36.32 ±1.05	36.07 ±0.98	36.13 ±0.89	36.06 ±1.11

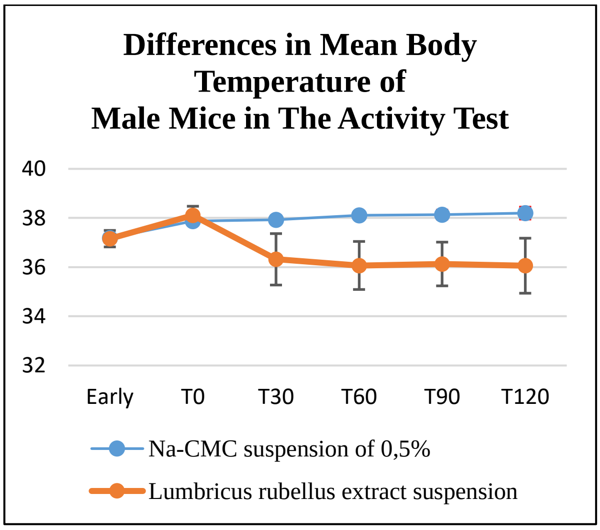

Figure 2. Graph of the mean body temperature of mice in the activity test in 2023

The specific alkaloids in earthworms (*Lumbricus rubellus*) that have antipyretic activity are not yet known with certainty, so further research is needed. The significance of the antipyretic activity of earthworms was proven through hypothesis testing.

Hypothesis testing of changes in body temperature of male mice was carried out to see the significance of the effect of giving earthworm extract suspension to the test group. The statistical test results can be seen in Table 2. Comparative tests were conducted to compare the antipyretic activity of earthworm extract with paracetamol. The dose of paracetamol given was 2 mg/20 gBB mice peroral. Based on the data obtained, the administration of paracetamol syrup can reduce the body temperature of mice. The results of statistical testing can be seen in Table 3. Comparative data on the mean body temperature of mice in the administration of paracetamol syrup and *Lumbricus rubellus* extract suspension can be seen in Table 4 and Figure 3. Hypothesis testing is used to determine the significance of differences in body temperature of mice after being given the intervention. The results of statistical testing can be seen in Table 5.

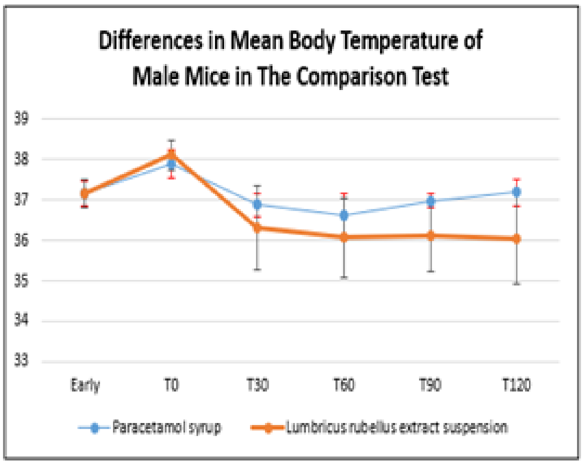

Figure 3. Graph of the mean body temperature of mice in the comparative test 2023

Table 2. Statistical test results in the activity test

Tn	Kruskal-Wallis Test	Post Hoc Comparison T_0 to T_n	Mean Rank
T30	p = 0.000	p = 0.000	18.83
T60		p = 0.000	17.67
T90		p = 0.000	18.28
T120		p = 0.001	19.61

Table 3. Statistical test results of paracetamol

Tn	Kruskal-Wallis Test	Post Hoc Comparison T_0 to T_n	Mean Rank
T30	p = 0.000	p = 0.000	16.61
T60		p = 0.000	12.00
T90		p = 0.001	19.28
T120		p = 0.022	26.50

Table 4. Mean body temperature of mice in the comparative test

Intervention	Average of Temperature (°C)					
	Early	T0	T30	T60	T90	T120
Paracetamol syrup	37.16 ±0.30	37.89 ±0.34	36.87 ±0.28	36.63 ±0.51	36.97 ±0.17	37.18 ±0.32
Lumbricus rubellus extract suspension	37.15 ±0.34	38.11 ±0.37	36.32 ±1.05	36.07 ±0.98	36.13 ±0.89	36.06 ±1.11

Table 5. Significance results of differences in body temperature of mice after intervention

Group (T60)	Mann-Whitney Test	Mean Rank
Earthworm extract suspension	0.017	6.50
Paracetamol syrup		12.50

The results of statistical testing with the Kruskal-Wallis non-parametric test show a significance value of p = 0.000 (Table 2) so that H0 is rejected and Ha is accepted, which means that there is a difference in the average body temperature of male mice (*Mus musculus*) induced by peptone before and after being given a suspension of earthworm extract (*Lumbricus rubellus*) at 30; 60; 90; 120 minutes. Then the Post Hoc follow-up test was conducted to determine the most significant temperature changes between the 30th, 60th, 90th, and 120th minutes. Based on the results of the Post Hoc follow-up test, it was found that there was no significant difference in the mean body temperature of mice when compared between the 30, 60, 90, and 120 minutes. However, based on the Mean Rank results obtained at the 60 minutes after giving the earthworm extract suspension has the lowest value, which is 17.67. This value means that the highest decrease in mice body temperature occurs at the 60 minutes after administration of earthworm extract suspension. Further research is needed to see the pharmacokinetic profile like the highest concentration of drug in specified compartment (C_{Max}) and time drugs need to clear from the highest concentration to half of its level ($T_{1/2}$) of the earthworm extract suspension to ensure its effectiveness. Data on the decrease in body temperature of mice after being given a suspension of earthworm extract at the 60 minutes was then used to conduct a comparative test against paracetamol syrup.

Absorption of paracetamol occurs rapidly and almost completely in the gastrointestinal tract, particularly in the small intestine. Absorption of paracetamol is not influenced by food and reaches peak concentrations in the blood relatively quickly after ingestion. Once absorbed, paracetamol is distributed throughout the body via the bloodstream. Most paracetamol is distributed in the extracellular fluid and only a small amount enters the cells. Paracetamol metabolism occurs in the liver. Most paracetamol undergoes conjugation with glucuronic and sulfuric acids, forming metabolites that are more easily excreted through the kidneys. However, a small amount of paracetamol also undergoes metabolism by cytochrome P450 enzymes, especially CYP2E1, forming a toxic metabolite (N-acetyl-p-benzoquinone imine) which is then neutralized by glutathione. The conjugated metabolites of paracetamol are excreted mainly through urine. Only a small portion is excreted through bile. The elimination half-life of paracetamol is generally short but may be prolonged in cases of overdose or individuals with impaired liver function [4, 5, 18].

Data were then analyzed using the Kruskal-Wallis non-parametric test (Table 3). The significance value of p = 0.000 was obtained, which means that there is a significant difference in mice body temperature after the administration of paracetamol syrup at the 30th, 60th, 90th, and 120th minutes. Then the Post Hoc follow-up test was carried out to determine the most significant temperature changes between the 30th, 60th, 90th, and 120th minutes. The results showed that there was a significant difference between the 60th and 120th minutes after the administration of paracetamol with a significance value of p = 0.019. Mean Rank data shows that at the 60th minute has a value of 12.00 which means there is the greatest decrease in temperature. At the 120th minute has a Mean Rank value of 26.50 which means the temperature drop is the smallest. This is to the theory that the time drug needs to reach its highest concentration after administration (T_{max}) of paracetamol is about 30-60 minutes while its half-life ($T_{1/2}$) is two hours.

Based on the body temperature measurement results (Figure 3 and Table 4), earthworm extract can decrease body temperature to a greater extent than paracetamol. To determine the significance of the temperature decrease that occurred, a hypothesis was carried out by using 60 minutes after paracetamol intervention as a comparison. Mann-Whitney testing shows a significance value of p = 0.017 so that H0 is rejected and Ha is accepted (Table 5), which means that there is a difference in body temperature of peptone-induced mice after administration of earthworm extract suspension and paracetamol at the minute with the highest decrease. This is evidenced by the Mean Rank value of earthworm extract suspension which is 6.50 while paracetamol syrup is 12.50. So, it can be stated that at the 60 minutes, giving a suspension of earthworm extract can reduce the body temperature of mice better than paracetamol syrup.

Earthworms have been known to contain bioactive compounds such as omega-3 fatty acids [20, 21], which have potential anti-inflammatory properties [22]. Peptone, which is a protein mixture resulting from protein hydrolysis, may have triggered an inflammatory response in the mice [21, 23]. Suspension of earthworm extract may have the effect of relieving inflammation, which can lead to a decrease in body temperature [24].

Earthworm extract may also affect the immune system of mice [23, 25]. Changes in the immune response may affect the production or release of substances that affect body temperature, such as prostaglandins [11]. A decrease in prostaglandin production may result in a decrease in body temperature. Earthworm extract may affect thermal regulation mechanisms in the body of mice. This may include its effect on vascular smooth muscle activity, which may affect blood flow and body temperature [10]. The earthworm extract suspension may also contain antioxidant compounds. Peptone induced in mice can increase oxidative stress, which can lead to changes in body temperature [26, 27]. The antioxidants in earthworm extract may help protect the mice's body from oxidative stress damage. There may be chemical interactions between the components in the earthworm extract and the induced peptone. This interaction may alter the chemical reactions in the mice's body which in turn affects body temperature [28, 29].

The in vivo study of earthworm extract on peptone-induced mice found encouraging results. What was originally earthworm treatment for typhoid fever only, now scientifically proven in vivo can reduce the temperature for non-typhoid fever. This research’s results are just a preliminary, it is necessary to test the treatment at the human level so that it can be used as a traditional medicine.

4. CONCLUSIONS

Statistical testing states a p-value = 0.000 which means there is a significant difference in the average body temperature of male mice (*Mus musculus*) induced by peptone before and after being given a suspension of earthworm extract (*Lumbricus rubellus*) at 30, 60, 90, 120 minutes. Based on statistical results, it can be stated that the suspension of earthworm extract has antipyretic activity. Statistical testing states the value of p = 0.017 which means there is a significant difference in body temperature of male mice (*Mus musculus*) induced by peptone after giving a suspension of earthworm extract (*Lumbricus rubellus*) and paracetamol at the minute with the highest decrease. The body temperature of mice at T60 after administration of earthworm extract suspension and paracetamol syrup had the highest decrease based on statistics. After the two were compared, it was found that the suspension of earthworm extract could reduce the body temperature of mice better than paracetamol syrup.

In development efforts, research needs to be carried out on the isolation results of earthworm (*Lumbricus rubellus*) alkaloids that have antipyretic activity, mechanism of action, and possible side effects. Secondly, it is necessary to research the onset and duration of action of peptone using various dose concentrations and injection methods. Third, studies on the toxicity of earthworms (*Lumbricus rubellus*) also need to be conducted. It would be beneficial to briefly discuss the potential risks and safety concerns related to the use of earthworm extract based on existing literature, if available. Fourth, research needs to be done on the immunomodulatory activity of earthworms (*Lumbricus rubellus*) associated with coelomycetes compounds. Fifth, research needs to be done on the pharmacokinetic profile of earthworms (*Lumbricus rubellus*).

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